Supplemental Information

for

A Paramagnetic Chemical Exchange-based MRI Probe Metabolized by Cathepsin D: Design, Synthesis and Cellular Uptake Studies

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Contents:

Purification and Isolation of Compound **6a**

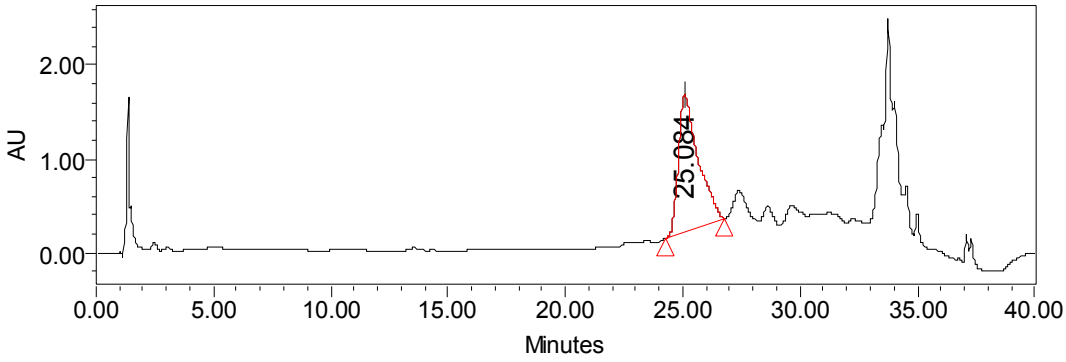


Figure 1: HPLC chromatogram of the crude mixture containing conjugate **6a,** t_R = 25.1 min.

Mass Spectral Characterization of Metalated Conjugates

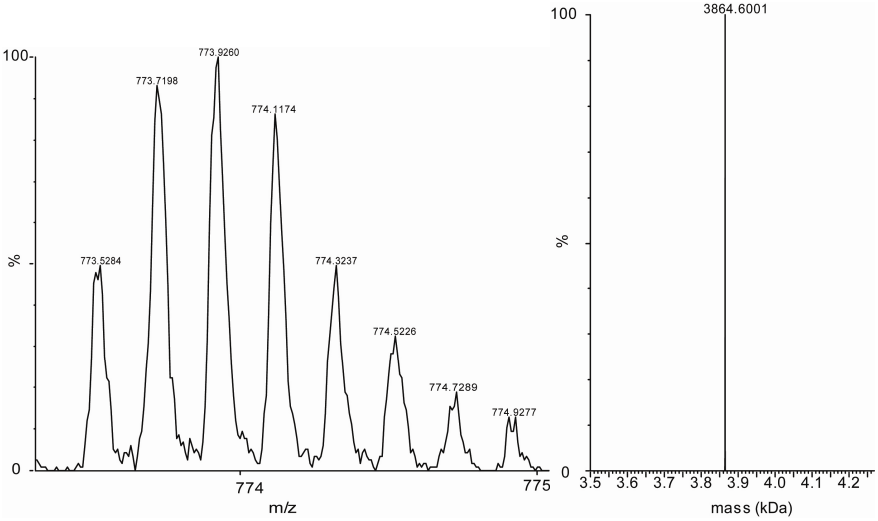


Figure 2: High resolution mass spectrum (ESI-TOF) of the probe **1** (M^{5+}, left). Deconvoluted (MaxEnt 1) spectrum of **1** (right). M=3864.6001, conforms to the formula: $C_{166}H_{249}F_2N_{53}O_{40}STm$

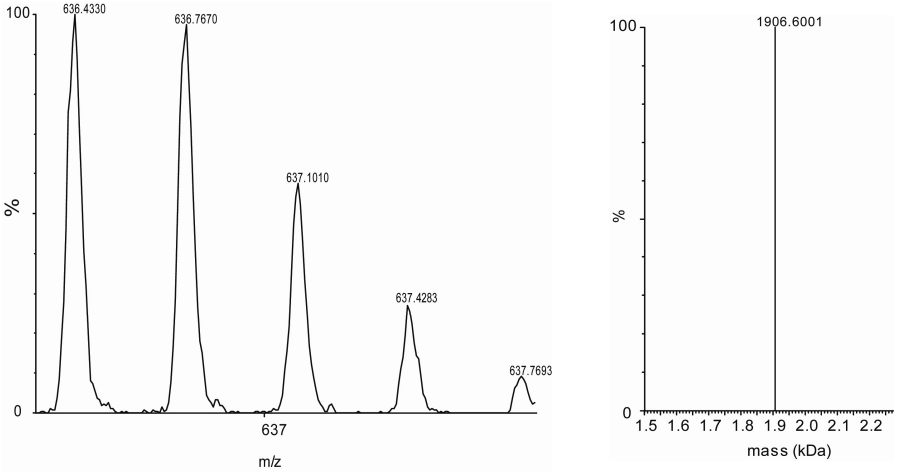
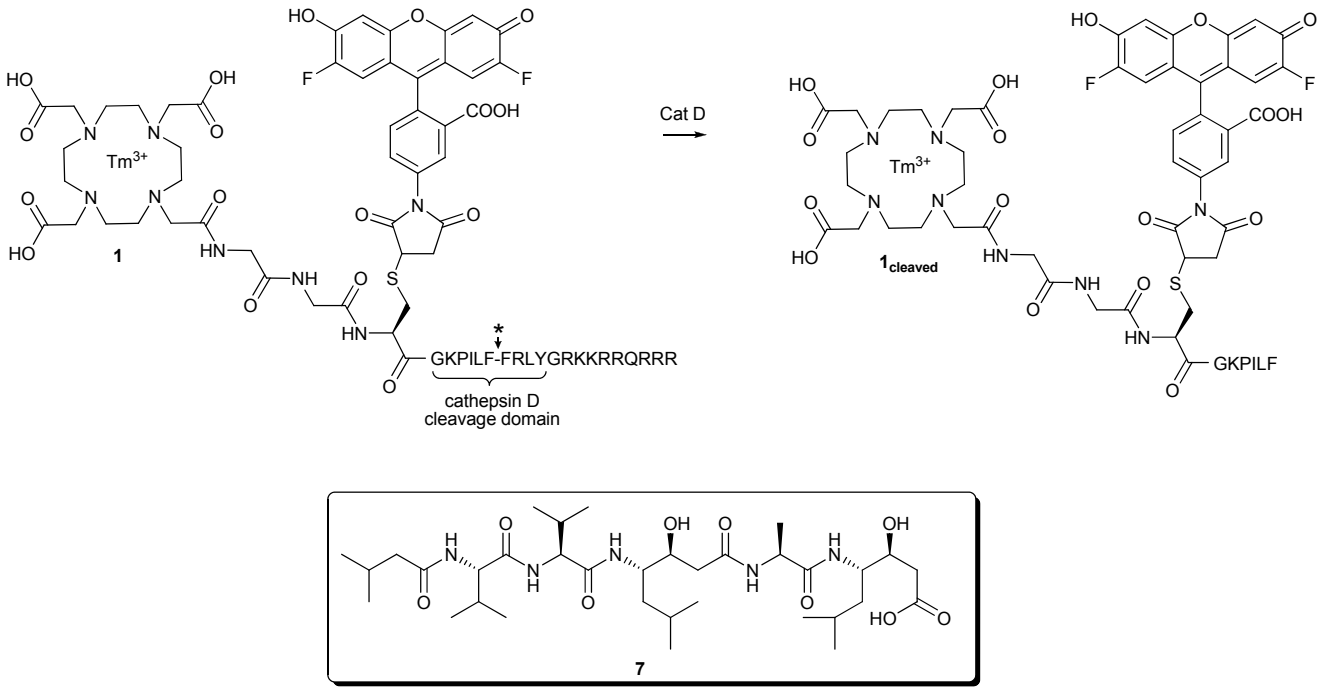


Figure 3: High resolution mass spectrum (ESI-TOF) of the fragment $\mathbf{1}_{\mathbf{cleaved}}$ (M^{3+}, left). Deconvoluted (MaxEnt 1) spectrum of $\mathbf{1}_{\mathbf{cleaved}}$ (right). M=1906.6001, conforms to the formula: $C_{81}H_{101}F_2N_{15}O_{24}STm$

In-vitro Metabolism of **1** by Cathepsin D.



Scheme 1: Metabolism of the dual probe **1** by cathepsin D (Cat D); hydrolysis occurs predominantly where indicated by the asterisk. Structure of pepstatin A (**7**), an inhibitor of Cat D.

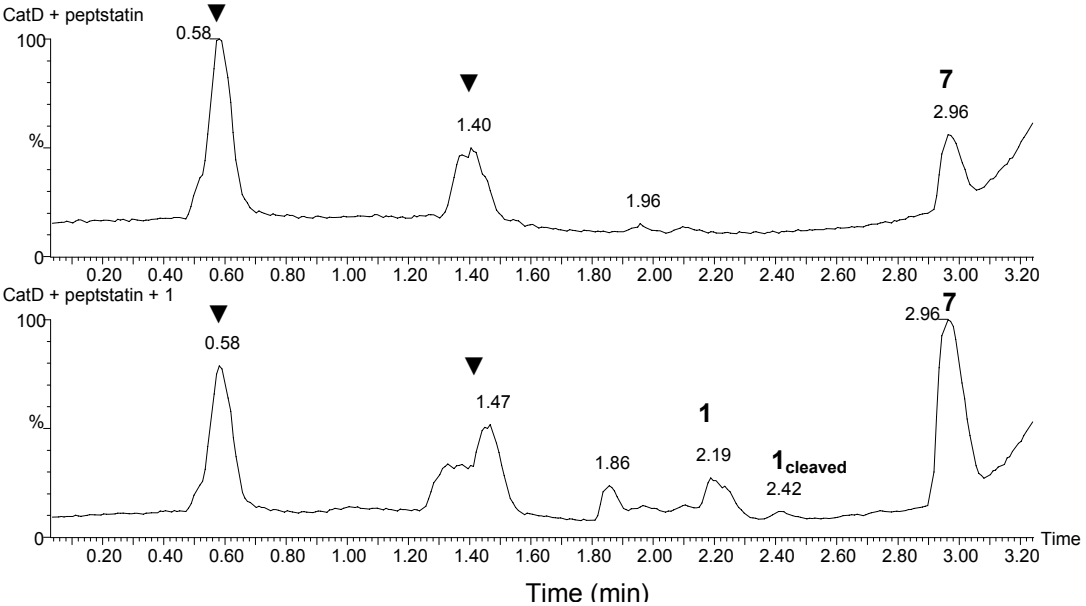


Figure 4: Total ion count spectrograms, positive mode ESI-TOF MS for the in-vitro metabolism of **1** by cathespin D. Top spectrogram: control sample containing cathepsin D (▼) in the presence of pepstatin

A (**8**) (t_R 2.96 min). Bottom spectrogram: cleavage of probe **1** (t_R 2.19 min) to yield fragment $\mathbf{1}_{\mathbf{cleaved}}$ (t_R 2.42 min).

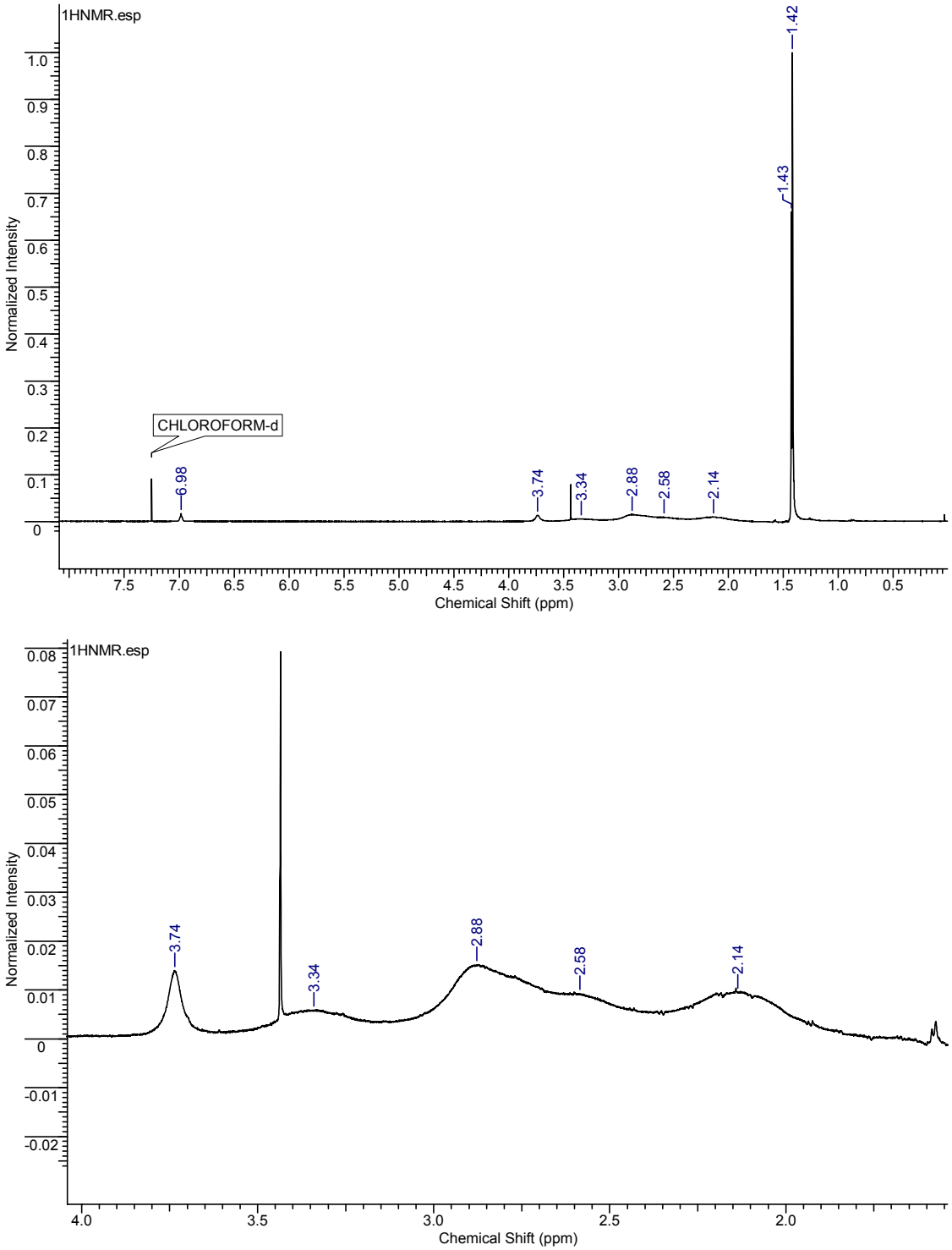


Figure 5: ^{1}H NMR spectrum of **4** in $CDCl_3$, full view (top) expanded region 1-4 ppm (bottom), showing extensive line broadening due to conformational rigidity.